IGF-1 ELISA Analysis

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Tradename: AC ExoRoot

Code: 60202

CAS #: 7732-18-5 & 91079-57-1 (or) 223749-83-5 & 123465-35-0 (or) 8002-43-5 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 13340

Lot #: N250520B

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:

Insulin-Like Growth Factor (IGF)-1 Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

Insulin-Like Growth Factor-1 (IGF-1) is a 70 amino acid polypeptide that plays a large role in mediating the actions of growth hormones. In addition to regulating mitosis, cell cycle, and apoptosis, IGF-I enhances hair follicle growth, maintains the anagen stage, and postpones the catagen stage. Specifically, increasing IGF-1 concentrations stimulates dermal papilla cells and the hair follicle, thus resulting in follicle elongation and hair growth.

Accordingly, an *in vitro* Insulin-Like Growth Factor (IGF)-1 ELISA was conducted to evaluate the ability of **AC ExoRoot** to alter IGF-1 levels in Human Dermal Papilla Cells. The key active ingredient in **AC ExoRoot**, *Chlorella vulgaris* Extract, was tested to demonstrate the superior nature of bioauthentic exosomes as a delivery system. Additionally, minoxidil and a blend of **AC ExoRoot** with minoxidil were tested to elucidate any interactive effects.

Assay Principle

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human IGF-1. Monoclonal antibodies specific for IGF-1 epitopes are coated on a microtiter plate. In positive samples, IGF-1 will bind to these antibodies and are tagged a second time with another IGF-1-specific antibody labeled with horseradish peroxidase (HRP). The addition of the chromagen solution, containing 3,3',5,5'-tetramethylbenzidine, provides the colorimetric reaction with HRP that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve provides a reference from the OD readings for the amount of IGF-1 in each sample.

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Materials

A. Kit:	IGF-1 ELISA Kit (Abcam; ab108873)*
B. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity (RH)
C. Equipment:	Forma Humidified Incubator; ESCO Biosafety Laminar Flow Hood; Synergy HT Microplate Reader; Pipettes; Light Microscope
D. Cell Line:	Human Hair Follicle Dermal Papilla Cells (HFDPCs) (Cell Applications Inc; 602K-05a)*
E. Media/Buffers:	Dermal Papilla Growth Media (DPGM) (Cell Applications Inc.; C-26501)*; Collagen Coating Solution (Cell Applications Inc.; 125-100)*; Minoxidil (Millipore Sigma)
F. Reagents:	Ascorbic Acid 2-Glucose (AA2G) (34 µg/mL)*
G. Culture Plate:	Flat Bottom 24-Well Tissue Culture Treated Microplates*
H. Software:	Excel Analysis TookPak (Microsoft)
I. Other:	Sterile disposable pipette tips

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Human dermal papilla cells were seeded into collagen coated 24-well tissue culture plates and allowed to grow to confluency in Complete Media. Ascorbic Acid 2-Glucose (AA2G) was diluted to 34 µg/mL and utilized as a positive control. Additionally, a 0.02% solution of minoxidil and 0.01%, 0.02%, 0.05% and 0.1% concentrations of *Chlorella vulgaris* Extract, **AC ExoRoot**, and **AC ExoRoot** with 0.02% minoxidil were prepared in Complete Media. All conditions were added to the dermal papilla cells for three days, after which media was collected and utilized in the IGF-1 ELISA Kit (ab108873) according to the manufacturer's instructions.

Briefly, standards were prepared in concentrations ranging from 0 ng/mL to 96 ng/mL. 50 µL of standards, controls, and samples were added to appropriate wells. After a two-hour incubation, samples were aspirated, and all wells were washed with 1x Wash Buffer five times. 50 µL of 1X Biotinylated Insulin like Growth Factor 1 Antibody was added to each well and incubated for two hours. The wash step, as described above, was repeated then 50 µL of 1X SP Conjugate was added to each well. After a 30-minute incubation and repeated wash step, 50 µL of Chromogen Substrate was added to each well and incubated for 30 minutes. Lastly, 50 µL of stop solution was added to each well and optical density was read at 450 nm.

Assays were repeated three separate times with each sample run in duplicate. Duplicates for each replicate were averaged and the average of all three experiments is displayed. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. A standard curve was created by reducing the data and generating a linear curve fit, to extrapolate IGF-1 concentrations expressed in ng/mL.

Percent change is expressed relative to Baseline values and calculated by the following equation:

$$Percent\ Change\ (\%) = \frac{IGF1\ Concentration_{Sample} - IGF1\ Concentration_{Complete\ Media}}{IGF1\ Concentration_{Complete\ Media}} \times 100$$

Results

The data obtained from this study met criteria for a valid assay and the positive control performed as anticipated. Compared to untreated cells, AA2G (34 µg/mL), increased IGF-1 production. Similarly, dermal papilla cells exposed to minoxidil, *Chlorella vulgaris* Extract, **AC ExoRoot**, and **AC ExoRoot** with minoxidil all demonstrated increases in IGF-1 production compared to untreated cells.

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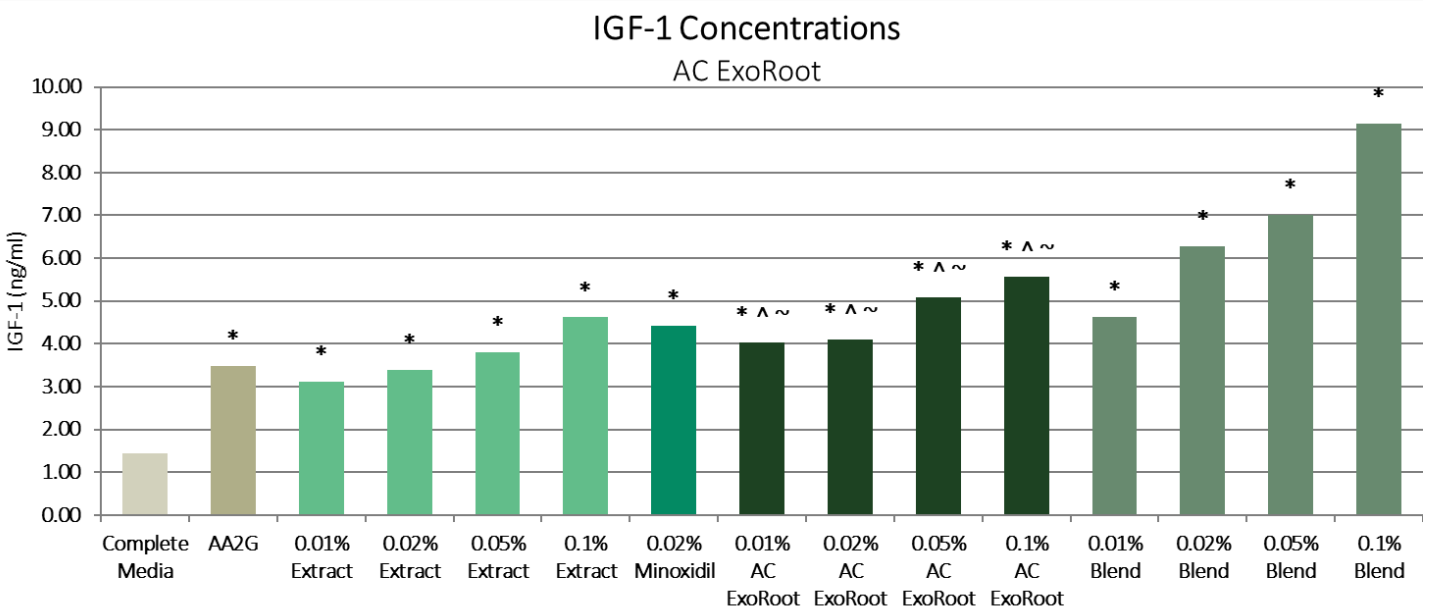

Figure 1. The Effect of **AC ExoRoot** on IGF-1 Production by Dermal Papilla Cells. Extract: *Chlorella vulgaris* Extract. Blend: % **AC ExoRoot** + 0.02% minoxidil. * indicates significance ($p < 0.05$) compared to untreated cells. ^ indicates significance ($p \leq 0.05$) compared to *Chlorella vulgaris* Extract. ~ indicates significance ($p \leq 0.05$) compared to **AC ExoRoot** + 0.02% minoxidil.

Table 1. Results from one-way ANOVA Statistical Analysis Compared to Untreated Cells. Extract: *Chlorella vulgaris* Extract. Blend: % **AC ExoRoot** + 0.02% minoxidil. * indicates significance ($p \leq 0.05$) compared to untreated cells.

	AA2G	0.01% Extract	0.02% Extract	0.05% Extract	0.1% Extract	0.02% Minoxidil	0.01% **AC ExoRoot**	0.02% **AC ExoRoot**	0.05% **AC ExoRoot**	0.1% **AC ExoRoot**	0.01% Blend	0.02% Blend	0.05% Blend	0.1% Blend
P Value	0.036*	0.047*	0.031*	0.023*	0.015*	0.017*	0.021*	0.022*	0.014*	0.010*	0.007*	0.010*	0.007*	0.008*

Table 2. Results from one-way ANOVA statistical analysis between two conditions compared at equivalent use levels. ^ indicates significance ($p \leq 0.05$) compared to *Chlorella vulgaris* Extract. ~ indicates significance ($p \leq 0.05$) compared to **AC ExoRoot** + 0.02% minoxidil.

	0.01% **AC ExoRoot**	0.02% **AC ExoRoot**	0.05% **AC ExoRoot**	0.1% **AC ExoRoot**
Chlorella vulgaris Extract	0.002^	0.025^	0.048^	0.019^
AC ExoRoot + 0.02% minoxidil	< 0.001~	0.016~	0.012~	0.028~

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Discussion

As shown in Figure 1, dermal papilla cells exposed to AA2G exhibited a 141% increase in IGF-1 levels compared to untreated cells. This data demonstrates the production of IGF-1 in dermal papilla cells is dynamic and can be manipulated with exogenous compounds.

Similarly, cells treated with 0.01%, 0.02%, 0.05% and 0.1% **AC ExoRoot** demonstrated 180%, 185%, 253%, and 287% increases in IGF-1 concentrations compared to untreated cells, respectively (Figure 1, Table 1). Comparatively, *Chlorella vulgaris* Extract at 0.01%, 0.02%, 0.05% and 0.1% only elicited increases of 117%, 135%, 165%, and 221% compared to untreated cells, respectively, and was significantly less effective than **AC ExoRoot** highlighting the superior nature of the bioauthentic exosomes as a delivery system (Table 2). These data demonstrate **AC ExoRoot** augments IGF-1 production in dermal papilla cells in a dose-dependent fashion.

Similarly, cells treated with 0.02% minoxidil exhibited 207% increases in IGF-1 concentrations compared to untreated cells (Figure 1; Table 1). Moreover, cells treated with 0.01%, 0.02%, 0.05% and 0.1% **AC ExoRoot** with 0.02% minoxidil exhibited 221%, 337%, 387%, and 536% increases in IGF-1 concentrations compared to untreated cells, respectively. At all concentrations, the blend exhibited a significant synergistic effect compared to **AC ExoRoot** alone (Table 2). These data indicate **AC ExoRoot** can be used in conjunction with minoxidil to stimulate IGF-1 production and boost hair growth.

Taken together, these results indicate **AC ExoRoot** augments IGF-1 production in dermal papilla cells. Collectively, **AC ExoRoot** elicits an environment known to stimulate hair elongation and maintain the anagen phase, promoting the growth of hair and hair follicles.